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Analgesic activity of aqueous and ethanolic extract of *Hemedesmus indicus* L. leaves and stem

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Abstract

Hemidesmus indicus L. (Family: Apocynaceae), is commonly referred as Indian sarsaparilla, Anantamool or Nannari, commonly available perennial climbing plant had been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to find the effect of aqueous and ethanolic extract of leaves and stem of *Hemidesmus indicus* for its analgesic activity. The analgesic activity was evaluated using models viz., acetic acid induced writhing response & eddys hot plate. Oral administration of the extract at the doses 200 and 400 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in ($p < 0.05$). Hence, present investigation established pharmacological evidences to support the folklore claim that *Hemidesmus indicus* is used as analgesic in reducing the pain.

Keywords: *Hemidesmus indicus*, Leaves, Stem, Analgesic activity

Introduction

Algesia (pain) is an ill-defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus. An analgesic selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. Analgesic activity means capacity of a substance to neutralize the pain sensation. Many medicines of plant origin had been used since long time without any adverse effects. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations[1]. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation for a particular disease[2].

Hemidesmus indicus L. (Family: Apocynaceae), is commonly referred as Indian sarsaparilla, Anantamool or Nannari, commonly available perennial climbing plant. It is used as main ingredient in the preparation of the cool and refreshing drink Nannari sharbat. Its native is India, also found in south tropical Asian countries such as Sri Lanka & Pakistan.

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The plant is used by the various tribal communities of India in the treatment of various disease and disorders[3-4], keeping this view the present work was conceived to explore the folk lore and traditional uses of this plant. As there is no reference in literature to the analgesic aspects, it was considered worthwhile to study the analgesic activity of aqueous and ethanolic extract leaves and stem of *Hemidesmus indicus*.

Material and Methods

Selection, collection and authentication of plant/plant material

The crude drug of *Hemidesmus indicus* L. was obtained from local area of Indore, M.P. and authenticated by Dr. S.N.Dwivedi, Professor and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P. (Voucher No. J/BOT/L-251).

Preparation of Extract (Leaves & Stem)

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered leaves & stem of *Hemidesmus indicus* L. (250gms) was loaded in Soxhlet apparatus and was extracted with ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.[5]

Animal

Adult rats of Wister strains of 150-250 grams and Swiss mice of either sex weighing about 18-25 grams have been obtained from local market of Indore. The Institutional Animal Ethical Committee approved the experimental protocols. The animals have been placed in a controlled room, with normal room temperature 25 ± 3^0C and humidity 35 - 50 %. Normal rat feeds and water *ad li betum* have been provided at regular interval of time. Animals have been housed in polypropylene cages. The animals have been allowed to acclimatize to laboratory conditions prior to experimental procedures.

Acute Toxicity Studies of Extracts

Acute oral toxicity studies have been conducted separately followed by using OECD guideline 423. The method used defined doses of 5, 50, 300, 2000 mg/kg *p.o.* body weight. Results were allowed substance rank and classify according to the Globally Harmonized System (GHS) for classification of chemicals which causes acute toxicity. From LD_{50} determination, $1/10^{th}$ of the dose was focused as the medial for pharmacological screening. Since all animals were alive & no toxicity and no significant changes in the body weight between the control and treated group were demonstrated at doses up to 2000 mg.[6]

Experimental design

Group	Category	Drug administered
1.	Normal Control	Normal saline or no any drug
2.	Positive control	standard drug as per activity
3.	Test 1 200 mg/kg	*H. indicus stem extract (ethanol)*
4.	Test 2 400 mg/kg	*H. indicus stem extract (ethanol)*
5.	Test 3 200 mg/kg	*H. indicus stem extract (Aqueous)*
6.	Test 4 400 mg/kg	*H. indicus stem extract (Aqueous)*
7.	Test 5 200 mg/kg	*H. indicus leaves extract (ethanol)*
8.	Test 6 400 mg/kg	*H. indicus leaves extract (ethanol)*
9.	Test 7 200 mg/kg	*H. indicus leaves extract (Aqueous)*
10.	Test 8 400 mg/kg	*H. indicus leaves extract (Aqueous)*

Analgesic activity[7-9]

Acetic acid - induced writhing response

Swiss albino mice were divided into 10 groups of 06 mice each (20-22 gm). Pentazocine (3mg/kg) *i.p.* was used as standard drug. The first group was given 10 ml/kg of normal saline orally, served as normal control and rest groups received drugs orally as per given experimental design. After 30 minutes of drug administration, mice of all groups were treated with Acetic acid (0.06% 1 ml acetic acid per 100 gm *i.p*). Then after five minutes of acetic acid injection mice were placed in individual cage and the number of abdominal contractions was counted for each mouse for a time period of 10 minutes.

Eddy's hot-plate

This test was carried out on a group of 06 Swiss mice of either sex (18-22 gm, n=6) using a Eddy's hot-plate apparatus. Only mice which showed initial nociceptive responses within 20 seconds were selected for the experiment. Animals were kept fasting of 16-18 hours. The extracts were dissolved in 2% gum acacia and administered orally. The reaction time (hind paw licking / jump response) of animals were delayed on hot plate maintained at 55 ± 1°C temperature was recorded & tabulated, after 30 minutes of drug administration. A cut off time was fixed of 10 seconds to avoid the injury to the paws. Diclofenac sodium (5 mg/kg), *p.o.* has been used as standard analgesic.

Statistical analysis

Results were tabulated and the data was expressed as mean ± SEM. The difference between experimental group were determined using one way analysis of variance (ANOVA) followed by Dunnet test. $P \leq 0.05$ was considered significant.

Results and Discussion

Aacute toxicity study by OECD guideline no. 423 for determination of $LD_{50.}$ This result indicates 200 mg/kg dose has been considered as effective dose (ED_{50}), for *H. indicus.*

Table 1: LD_{50} & ED_{50} of *H. indicus* Linn.

Plant Name	LD_{50}	ED_{50}
***H. indicus* L.**	2000 mg/kg	200 mg/kg

The aqueous and ethanolic extract of *H. indicus* L. leaves and stem were evaluated for analgesic activity in animal models and the results are summarized in Table 2 & 3. The results (acetic acid induced writhing) obtained indicates that the extract found to have significant ($P < 0.05$) analgesic activity in rats. The aqueous leaves extract at the test doses 400 mg/kg b.w. showed highest % inhibition of 84.24 and was found to be maximum as compared to standard

drug which showed 89.52% of inhibition while in case of eddy's hot plate aqueous stem extract at the dose of 400 mg/kg b.w showed 64.03 % as compared to standard which showed 71.0 %.

Table 2: Analgesic activity of *H. indicus* in acetic acid induced writhing method in mice

Group Dose mg/kg *(p.o.)*	Treatment (mg/kg), *p.o.*	No. of abdominal writhing (10 minutes)	% inhibition
Normal Control	Normal saline	21.0 ± 3.7	--
Positive control	Pentazocine 3 mg/kg	2.2 ± 1.5**	89.52
Test 1 200 mg/kg	Stem extract *(ethanol)*	8.8 ± 1.0*	58.09
Test 2 400 mg/kg	Stem extract *(ethanol)*	4.5 ± 2.3*	78.57
Test 3 200 mg/kg	Stem extract *(Aqueous)*	8.6 ± 5.3**	59.04
Test 4 400 mg/kg	Stem extract *(Aqueous)*	4.4 ± 3.3**	79.04
Test 5 200 mg/kg	Leaves extract *(ethanol)*	8.4 ± 2.3*	60.00
Test 6 400 mg/kg	Leaves extract *(ethanol)*	4.3 ± 5.4**	79.52
Test 7 200 mg/kg	Leaves extract *(Aqueous)*	7.9 ± 5.4*	63.28
Test 8 400 mg/kg	Leaves extract *(Aqueous)*	3.3 ± 2.1**	84.28

All values are mean ± SEM, n=6, *P<0.05 indicates significant and **P<0.001 is more significant when compared with control.

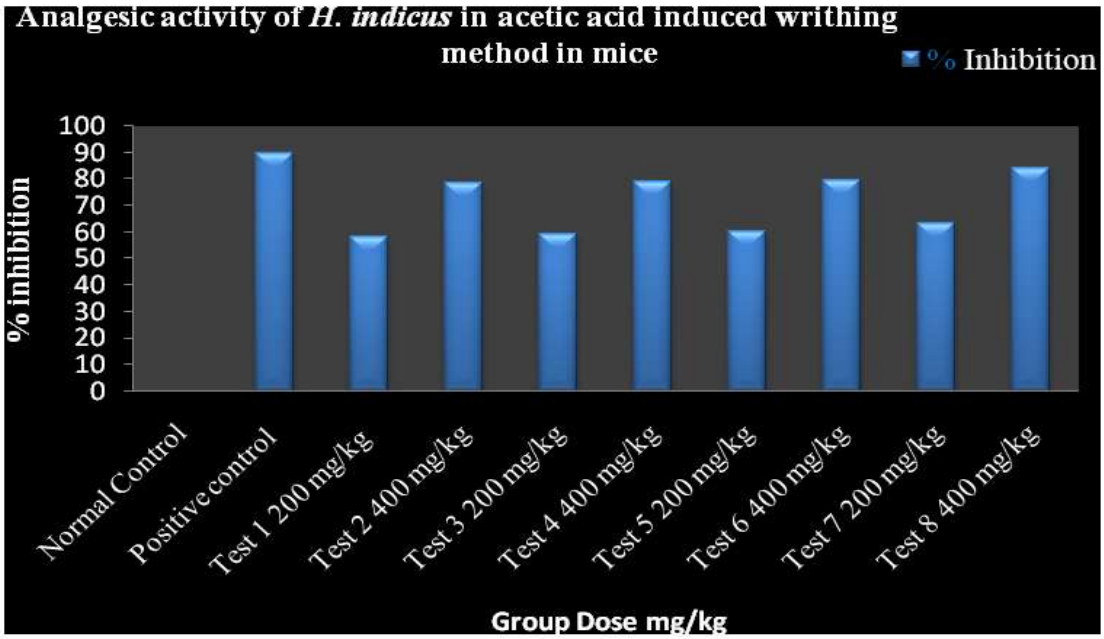
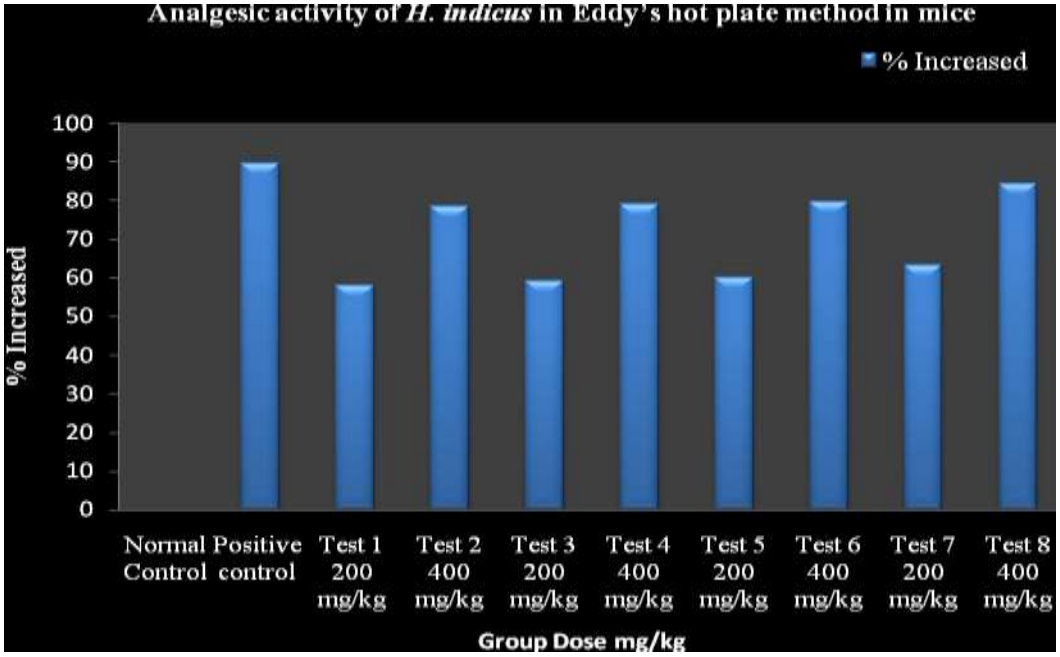


Table 3: Analgesic activity of *H. indicus* in Eddy's hot plate method in mice

Group Dose mg/kg *(p.o.)*	Treatment (mg/kg), *p.o.*	Jumping response (10 seconds)	% increased
Normal Control	Normal saline	8.37 ± 0.20	--
Positive control	Diclofenac sodium 5 mg/kg	2.43 ± 0.05**	71.00
Test 1 200 mg/kg	Stem extract *(ethanol)*	6.03 ± 0.19*	28.00
Test 2 400 mg/kg	Stem extract *(ethanol)*	3.44 ± 0.19*	59.00
Test 3 200 mg/kg	Stem extract *(Aqueous)*	7.00 ± 0.25*	19.57
Test 4 400 mg/kg	Stem extract *(Aqueous)*	3.01 ± 0.09**	64.03
Test 5 200 mg/kg	Leaves extract *(ethanol)*	5.25 ± 0.20*	37.27
Test 6 400 mg/kg	Leaves extract *(ethanol)*	3.16 ± 0.08**	62.24
Test 7 200 mg/kg	Leaves extract *(Aqueous)*	6.50 ± 0.06*	28.00
Test 8 400 mg/kg	Leaves extract *(Aqueous)*	3.15 ± 0.12**	62.36

All values are mean ± SEM, n=6, *P<0.05 indicates significant and **P<0.001 is more significant when compared with control.



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